# Prima RNApols™ ExTend kit

primrose bio™

**Part # PBLTK**

RNA polymerase kit for *in vitro* transcription (IVT) reactions.

**Description**
The Prima RNApols ExTend kit is designed to synthesize long (≥ 5 kb) mRNA products with high integrity and low dsRNA from IVT reactions.

**Use & Handling**
Store at -20°C.
For Research Use Only.

The kit contains the following:

| Kit Component | Part Number | Concentration |
|---|---|---|
| 2 kb Linearized DNA template | PBLT2-0.025 | 250 ng/µL |
| RNApols™ ExTend RNA Polymerase | PBRD3-0.1 | 50 U/µL |
| 5x IVT Reaction Buffer | PBB-0.1 | 5x |

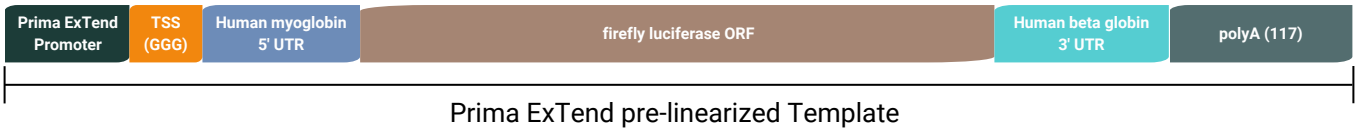
## 2 kb Linearized DNA Template Instructions for Use

### DNA Template design

Linearized plasmid DNA or PCR amplified DNA can be used as templates for IVT using the Prima ExTend kit.

The Prima ExTend kit comes with a purified, ready-to-use linearized plasmid (linearized using a restriction enzyme downstream of the poly(A) tail sequence) for use as a control template in an IVT reaction (Part # PBLT2-0.025).

The pre-linearized DNA template contains the Prima ExTend promoter, transcription start site (TSS), 5' untranslated region (UTR) sequence, firefly luciferase open reading frame, 3' UTR sequence, and a poly(A) sequence (Figure 1).




Prima ExTend pre-linearized Template
(2010 bp)

Figure 1: Linear DNA template included in the Kit (Part # PBLT2-0.025)

The Prima RNApol ExTend polymerase is only compatible with the Prima ExTend promoter. This sequence must be incorporated into the DNA template of interest to successfully generated mRNA during IVT. The Prima ExTend RNA polymerase does not recognize the T7 promoter sequence.

**Continued**

**Incorporation of Prima ExTend promoter sequence:**
The Prima ExTend promoter sequence can be incorporated into a DNA template by either PCR or through incorporation into plasmid DNA.

**Prima ExTend promoter sequence:**
TTGATTAATTAACCCACACTATAGGG
TSS

***PCR generated templates:***
For PCR generated DNA templates, we suggest incorporating the Prima ExTend promoter sequence directly upstream of the transcript to be generated. We recommend designing a forward primer that includes the promoter sequence plus an additional 20 bases of homology to your UTR sequence (Figure 2). Perform a DNA cleanup step and verify the construct for accuracy before running an IVT reaction.

***Linearized Plasmid DNA:***
For templates larger than 5 kb, we suggest incorporating the promoter into a plasmid directly upstream of the transcript to be generated using seamless molecular cloning techniques.
The plasmid DNA should be linearized by restriction enzyme digestion downstream of the transcript encoding region. Perform a DNA cleanup step and verify the construct for accuracy before running an IVT reaction.

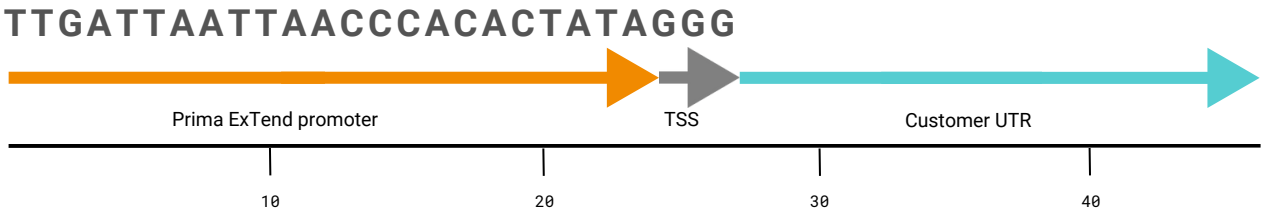



Figure 2: Recommended primer design to incorporate Prima ExTend Promoter sequence

## Prima ExTend IVT Reaction Instructions for Use

**Required reagents**
**NOTE: All reagents must be RNase-free. Use recommended source or equivalent grade.**

### Reagents included in the Prima RNApols ExTend kit:

2 kb Linearized DNA template (Part # PBLT2-0.025) or DNA template with Prima ExTend promoter sequence incorporated. Sufficient DNA template is supplied for 10 control IVT reactions.

Prima RNApol ExTend RNA polymerase (Part # PBRD3-0.1)

5x IVT Reaction Buffer (Part # PBB-0.1)

### Reagents not included in the kit:

Nucleoside-5'-Triphosphate (NTP) Set (Primrose Bio recommends the Thermo Scientific NTP Set (100 mM), Part # R1481)

Cap Analog (if being used in IVT reaction)

Inorganic pyrophosphatase (Recommended) (Primrose Bio recommends Thermo Scientific, Part # EF0221)

RNase inhibitor (Optional) (Primrose Bio recommends Thermo Scientific, Part # EO0382)

**Continued**

## Reagent Preparation and Protocol

**RNase-free techniques**
Ensure all reagents used are RNase free. Use disposable RNase-free tubes and bottles. When possible, use dedicated RNase-free pipettes. Avoid using pipettes that have been used for plasmid preparation using RNase A. Reactions should be assembled nuclease-free in microcentrifuge tubes, PCR strip tubes or 96-well plates.

**5x Reaction Buffer**
The 5x IVT Reaction Buffer is specifically formulated for the Prima ExTend RNA polymerase to maximize yield and integrity while minimizing dsRNA impurities in the mRNA produced.

Table 1: IVT Reaction Conditions for the Prima RNApol ExTend kit

| REAGENT | AMOUNT | FINAL CONCENTRATION |
|---|---|---|
| Nuclease-free water | Add water up to a final IVT volume of 20 µL | -- |
| 5x Reaction Buffer | 4 µL | 1 x |
| ATP (100 mM) | 1 µL | 5 mM |
| GTP (100 mM) | 1 µL | 5 mM |
| UTP (100 mM)[1] | 1 µL | 5 mM |
| CTP (100 mM) | 1 µL | 5 mM |
| Cap analog (100 mM)[2] | 0.8 µL | 4 mM |
| RNase inhibitor (40 unit/µl)[3] | 0.5 µL | 1 U/µL |
| Inorganic Pyrophosphatase (0.1 U/µl)[4] | 0.4 µL | 0.002 U/µL |
| Template DNA[5] | 1.7 µL (450 ng) | 8 nM |
| Mix briefly | | |
| Prima RNApol ExTend RNA polymerase | 2 µL | 100 Units |
| **Total reaction volume** | **20 µL** | -- |
| Mix briefly | | |

1. *Pseudo-UTP or N1-Methylpseudouridine-5'-Triphosphate can be substituted for UTP as desired.*
2. *Addition of cap analog (not provided in the kit) to the reaction is optional.*
3. *Addition of RNase inhibitor (1 U/µL final, not provided in the kit) to the reaction is optional but recommended.*
4. *Addition of inorganic pyrophosphatase (0.002 U/µL final, not provided in the kit) to the reaction is highly recommended.*
5. *Volume to be added to the IVT is based upon the 2 kb Linearized DNA template (Part # PBLT2-0.025, provided at 250 ng/µL). This value may change dependent upon the concentration of the DNA template used.*

**Continued**

**Transcription reaction**

1. Thaw 5x IVT buffer, NTPs, linear DNA template and cap analog at room temperature. Mix and pulse-spin in microfuge to collect solutions to the bottom of tubes. Keep at room temperature while in use.
2. Place the Prima ExTend RNA polymerase, RNase inhibitor (optional), and inorganic pyrophosphatase (recommended) on ice.
3. Assemble the IVT reaction at room temperature in the order shown in Table 1. The mixture can be scaled dependent upon the number of IVT reactions and the volume of the reaction required.
4. Upon addition of the DNA template to the IVT reaction, mix the solution briefly. Add the Prima ExTend RNA polymerase as the final component to the IVT reaction mixture. Seal the IVT reaction mixture and incubate the IVT reaction at 37°C for 2 hours. We recommend using a dry air incubator or thermocycler, to prevent evaporation. A water bath may also be used.

## Post-IVT Reaction Considerations

The Prima RNApol ExTend kit is designed to generate high concentrations of mRNA. As a result, the reaction mixture can become viscous. Dilution of the IVT reaction mixture may be required after treatment with DNase I.
The mRNA generated by the Prima RNApol ExTend kit may be purified by any traditional method such as lithium chloride precipitation or plate-based purification.

**5' Post-transcriptional capping**

The mRNA generated from the Prima RNApols™ ExTend Kit is compatible with the use of post-transcriptional capping systems.